



## Research article

Application and optimization of ultrasound-assisted deep eutectic solvent for the extraction of new skin-lightening cosmetic materials from *Ixora javanica* flowerNina Dewi Oktaviyanti<sup>a,b</sup>, Kartini<sup>b</sup>, Abdul Mun'im<sup>a,c,\*</sup><sup>a</sup> Department of Pharmacognosy-Phytochemistry, Faculty of Pharmacy, Universitas Indonesia, Cluster of Health Sciences Building, Depok, 16424, West Java, Indonesia<sup>b</sup> Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Surabaya, Surabaya, 60293, East Java, Indonesia<sup>c</sup> Graduate Program of Herbal Medicine, Faculty of Pharmacy, Universitas Indonesia, Kampus UI, Depok, 16424, West Java, Indonesia

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## ABSTRACT

The high demand for cosmetics has had a great impact on the development of innovative products in the cosmetic industry. The availability of raw materials has become a common problem in the cosmetic industry. Materials from nature can act as alternative sources, such as *Ixora javanica*. Several studies have shown the potential of *I. javanica* as an antioxidant and skin lightening agent. The objectives of the present study were to develop and optimize a green ultrasound-assisted deep eutectic solvent extraction of *I. javanica*. Eleven deep eutectic solvents were evaluated based on extraction efficiency parameters; that is, flavonoid and anthocyanin yields; the antioxidant and tyrosinase inhibitory activities of the extracts. The combination of choline chloride and propylene glycol (1:1) was shown to be the optimal deep eutectic solvent for *I. javanica* extraction. The extraction parameters of temperature, extraction time, and solid-to-liquid ratio were also optimized using response surface methodology. The total flavonoid compound obtained was 33 mg quercetin equivalent/g dried sample under the optimum extraction condition (extraction time of 5 min, temperature of 57 °C, solid-to-liquid ratio of 0.02 g/mL). In sum, this work demonstrates the potential of natural deep eutectic solvent as an organic solvent replacement to obtain high quality *Ixora javanica* extract, which is a potential new source of skin-lightening cosmetic materials.

## 1. Introduction

*Ixora javanica* is a shrub or small tree plant belonging to the family Rubiaceae. Because of its attractive and distinctive color, it is commonly known as “common red *Ixora*,” “jungle flame *Ixora*,” or “Soka Jawa” in Indonesia. It is very well known by the community and has been widely studied for its compounds and biological activities (Kharat et al., 2013). Extract activities reported in previous studies included antioxidant, antitumor, anti-inflammatory, and hepatoprotective effects (Nair et al., 1991; Hemalatha et al., 2012; Dontha et al., 2016; Vishwanadham et al., 2016). Studies have shown that the flower provides the greatest activity compared to the other parts of the plant (Rohini et al., 2012; Dontha et al., 2015).

Various polyphenolics, such as flavonoids and anthocyanins, were found to be the primary extract compounds in the *I. javanica* flowers (Dontha et al., 2015). Several studies showed that polyphenolics, flavonoid, and anthocyanin compounds were responsible for most of the

activity of *Ixora* flower extracts (Nair et al., 1991; Kharat et al., 2013; Dontha et al., 2015, 2016; Usha et al., 2016; Vishwanadham et al., 2016). Furthermore, polyphenolic, flavonoid, and anthocyanin compounds reportedly exhibit tyrosinase inhibitory activity (An et al., 2008; Chang, 2012; Liang et al., 2014). Methanolic extract from the *Ixora* flower has also shown activity as a tyrosinase inhibitor (Rohini et al., 2012). There are almost 500 species belonging in the genus *Ixora*. Compared to other species, *Ixora javanica* contain high level of ferulic acid and its derivatives (Nair et al., 1991; Kharat et al., 2013; Dontha et al., 2015; Usha et al., 2016). On the other hand, ferulic acid was known as tyrosinase inhibitor due to its structural similarity with tyrosine so that it can compete with tyrosine to occupy the active side of the tyrosinase (An et al., 2008; Liang et al., 2014).

Thus far, research on *Ixora* flowers was limited to compound extraction using organic solvents. However, several reports have shown the potential toxicity of organic solvents for both humans and the environment (De Carvalho and Da Fonseca, 2004; Levet et al., 2016; Lin et al.,

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2018; Seo and Kim, 2018). Environmental awareness has encouraged efforts to find safer and environmentally friendly alternative solvents (green solvents), including in the extraction process (green extraction). Deep eutectic solvents (DESs), which comprise hydrogen bond acceptors (HBAs) and hydrogen bond donors (HBDs) in certain molar ratios, are among the most popular green solvents because of their advantages over other solvents that include less toxicity, easy obtainability, and high extraction efficiency. In addition, the HBA and HBD combination can be adapted according to the extraction requirements (Pena-Pereira and Tobiszewski, 2017). Choline chloride as the HBD has been widely used in previous studies because it is highly effective in plant metabolite extraction (Ruesgas-Ramon et al., 2017).

The utilization of green extraction must be supported by the use of environmentally friendly extraction methods and minimal energy use. Ultrasound-assisted extraction (UAE) is a nonconventional extraction method mediated by ultrasonic waves that is often used in green extraction. The cavitation phenomenon in UAE can increase extraction efficiency so that the extraction time, solvent use, and energy consumption can be reduced (Rutkowska et al., 2017).

To our best knowledge, the application of green extraction using DESs in the *I. javanica* extraction process has not been reported elsewhere. This study aimed to determine environmentally friendly solvent alternatives for extraction of *I. javanica* that can provide high levels of flavonoid and anthocyanin compounds with antioxidant and tyrosinase inhibitory activity. The optimum parameters for the flavonoid extraction were also investigated.

## 2. Materials and methods

### 2.1. Chemicals

The chemicals used in this study included pharmaceutical grade choline chloride (Xi'an Rongsheng Biotechnology Co, Ltd, China); propylene glycol, glycerol, ethylene glycol, polyethylene glycol, sorbitol, 1,3-propanediol, oxalic acid, lactic acid, glycolic acid, malic acid, and citric acid (Merck, Germany); 2,2-diphenyl-1-picrylhydrazyl (DPPH), quercetin, mushroom tyrosinase, and L-tyrosine (Sigma Aldrich, USA).

### 2.2. Plant materials

Fresh *Ixora javanica* flowers were collected from Dusun Ngampel, Kediri, East Java, Indonesia, and were authenticated by the Center for Traditional Medicine Information and Development, Faculty of Pharmacy, University of Surabaya. Red flower blooms were collected, washed, and dried under shade. After shade drying, the flowers were mechanically powdered and sieved using a size 30 mesh. The dry powder was stored in an airtight container until further processing.

### 2.3. Preparation of deep eutectic solvents

DESs were prepared using a heating method. Choline chloride and the HBD were weighed and used at various molar ratios. The compounds were combined and constantly stirred at 50 °C for 30 min until a homogeneous transparent liquid was generated. Table 1 lists the choline chloride and HBD molar ratios and the abbreviations of the DESs used in this study.

### 2.4. UAE procedure

The solid-to-liquid ratio of 0.05-g dried flower powder per milliliter of DES or conventional solvent (ethanol) was used during extraction with the UAE method. Extraction was carried out at room temperature for 25 min. The extracts were centrifuged at 1500 rpm for 15 min and the filtrates were collected. The filtrate was adjusted to a final volume of 10.0 mL. The component yield and activities of the filtrate were then determined. All extraction procedures were conducted in triplicate.

**Table 1.** List of DESs and their abbreviation used in this study.

Abbreviation	Combination of HBA and HBD	Molar ratio
ChPg	Choline chloride: propylene glycol	1:1
ChGl	Choline chloride: glycerol	1:2
ChEg	Choline chloride: ethylene glycol	1:2
ChPeg	Choline chloride: polyethylene glycol	1:2
ChSb	Choline chloride: sorbitol	1:1
ChPd	Choline chloride: 1,3-propanediol	1:3
ChOa	Choline chloride: oxalic acid	1:1
ChLa	Choline chloride: lactic acid	1:2
ChGa	Choline chloride: glycolic acid	1:1
ChMa	Choline chloride: malic acid	1:1
ChCa	Choline chloride: citric acid	1:1

### 2.5. Determination of total flavonoid content in DES extracts

The total flavonoid content was determined by spectrophotometry according to the method described by Mun'im et al. (2017) with minor modifications. In brief, 1.0 mL of extract filtrate, 1.5 mL of 0.32%  $\text{AlCl}_3$ , and 1.5 mL of 10% sodium acetate solutions were mixed in a volumetric flask. The mixture then had 96% ethanol added until a total solution volume of 10.0 mL was obtained. The mixture was homogenized and incubated for 30 min. The absorbance of each sample was analyzed using a UV-Vis spectrophotometer (UV-1900, Shimadzu Corp, Kyoto, Japan) at  $\lambda_{\text{max}}$  of 432.5 nm. The reference compound standard was quercetin. The total flavonoid content (TFC) was expressed in terms of milligram quercetin equivalent (QE) per gram of dried flower powder (mg/g). The procedures were conducted in triplicate.

### 2.6. Determination of total anthocyanin content in DES extracts

The total anthocyanin content of each DES extract was analyzed using the pH differential spectrophotometric method of Lee et al. (2005) with modification. Initially, two sample solutions were prepared. One solution was prepared by diluting 1.0-mL extract filtrate with potassium chloride buffer and adjusting to pH 1.0 until a volume of 5.0 mL was obtained. The other solution was diluted with sodium citrate buffer and adjusted to pH 4.5. Each solution was homogenized and incubated for 20 min at room temperature. The absorbance of the pH-adjusted sample solutions were measured at 510 nm ( $A_{510}$ ) and 700 nm ( $A_{700}$ ) using a 1-cm path length cuvette. The total anthocyanin content (TAC) was calculated as milligrams monomeric anthocyanin of cyanidin-3-glucoside equivalent (CgE) per gram dried flower powder (mg/g) according to Eq. (1):

$$\text{TAC} = \frac{A \times \text{MM} \times \text{DF} \times 1000}{\epsilon \times l} \quad (1)$$

where A is  $(A_{510} - A_{700})_{\text{pH } 1.0} - (A_{510} - A_{700})_{\text{pH } 4.5}$  sample absorbance; MM is the molecular mass of cyanidin-3-glucoside (449.2 g/mol); DF is the dilution factor;  $\epsilon$  is the molar absorptivity of cyanidin-3-glucoside (26,900 L/cm-mol); 1 is the correction optic path factor (1 cm); and 1000 is the conversion of grams to milligrams.

All analyses were performed in triplicate.

### 2.7. In vitro antioxidant activity

The antioxidant activity of the extract was evaluated by its DPPH radical scavenging activity (Bakirtzi et al., 2016). Each diluted solution was mixed with 3.0 mL of 0.004% DPPH and incubated for 30 min. The absorbance of the extract mixture ( $A_{\text{extract}}$ ) and the absorbance of 0.004% DPPH ( $A_{\text{DPPH}}$ ) were determined at 517.0 nm. The DPPH scavenging

**Table 2.** Composition of solution in tyrosinase inhibitor assay.

Solution	Composition of solution (μl)			
	a	b	c	d
Phosphate buffer	120	160	80	120
Substrate (L-tyrosine)	40	40	40	40
Sample	-	-	40	40
Mushroom tyrosinase	40	-	40	-

**Table 3.** The coded, range, and real levels of each factor for the experimental design.

Factor	Unit	Code	Range and level (xi)		
			-1	0	1
Extraction time	min	x <sub>1</sub>	5	10	20
Temperature	°C	x <sub>2</sub>	30	40	57
Solid-to-liquid ratio	g/mL	x <sub>3</sub>	1:20	1:30	1:50

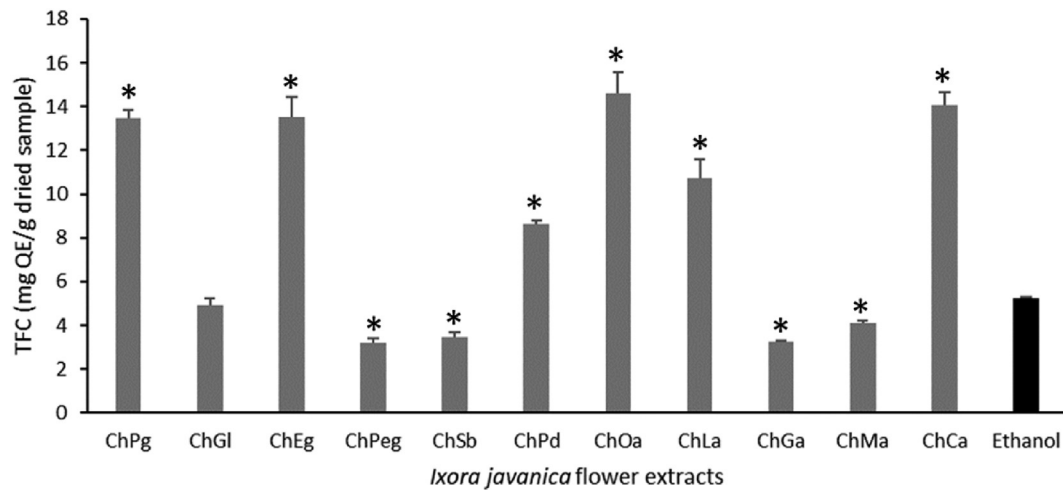
activity was calculated as percentage inhibition using Eq. (2). Quercetin was used as a positive control. The experiments were performed in triplicate.

$$\% \text{ inhibition of DPPH radical} = \frac{A_{DPPH} - A_{extracts}}{A_{DPPH}} \quad (2)$$

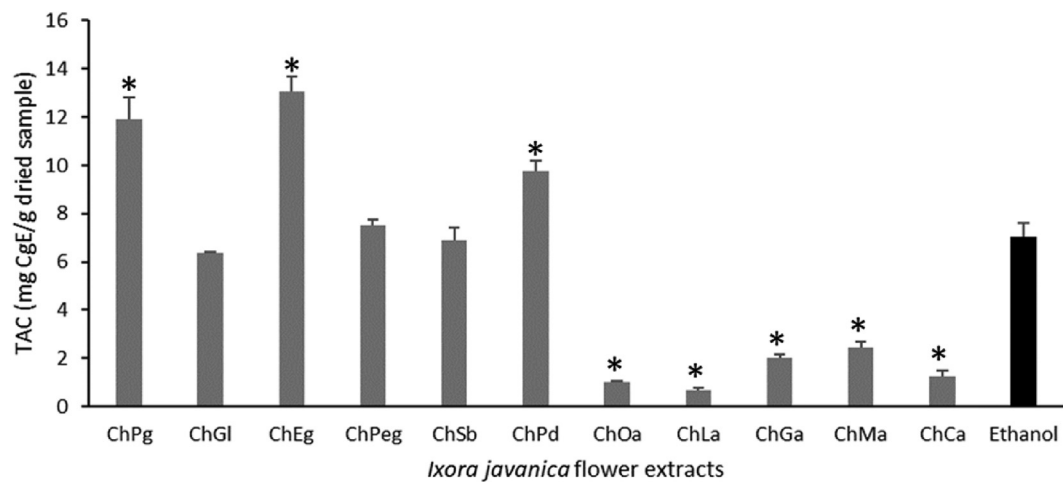
## 2.8. In vitro tyrosinase inhibitory activity

Assays were performed as previously described by Chiocchio et al. (2018) with slight modifications. Mushroom tyrosinase solution was freshly prepared from 1.73 mg of 500 U/mL mushroom tyrosinase diluted in 10.0 mL of 0.05 M phosphate buffer solution, pH 6.5. Substrate solution was also freshly prepared by diluting 1.81 mg of L-tyrosine in 10.0 mL of 0.05 M phosphate buffer solution. Each of the solutions and sample were mixed at certain volumes according to Table 2.

All of the mixtures were incubated at 25 °C for 30 min, and the reaction was monitored using a microplate reader at 475 nm. The percentage inhibition of tyrosinase activity was calculated using the following equation:

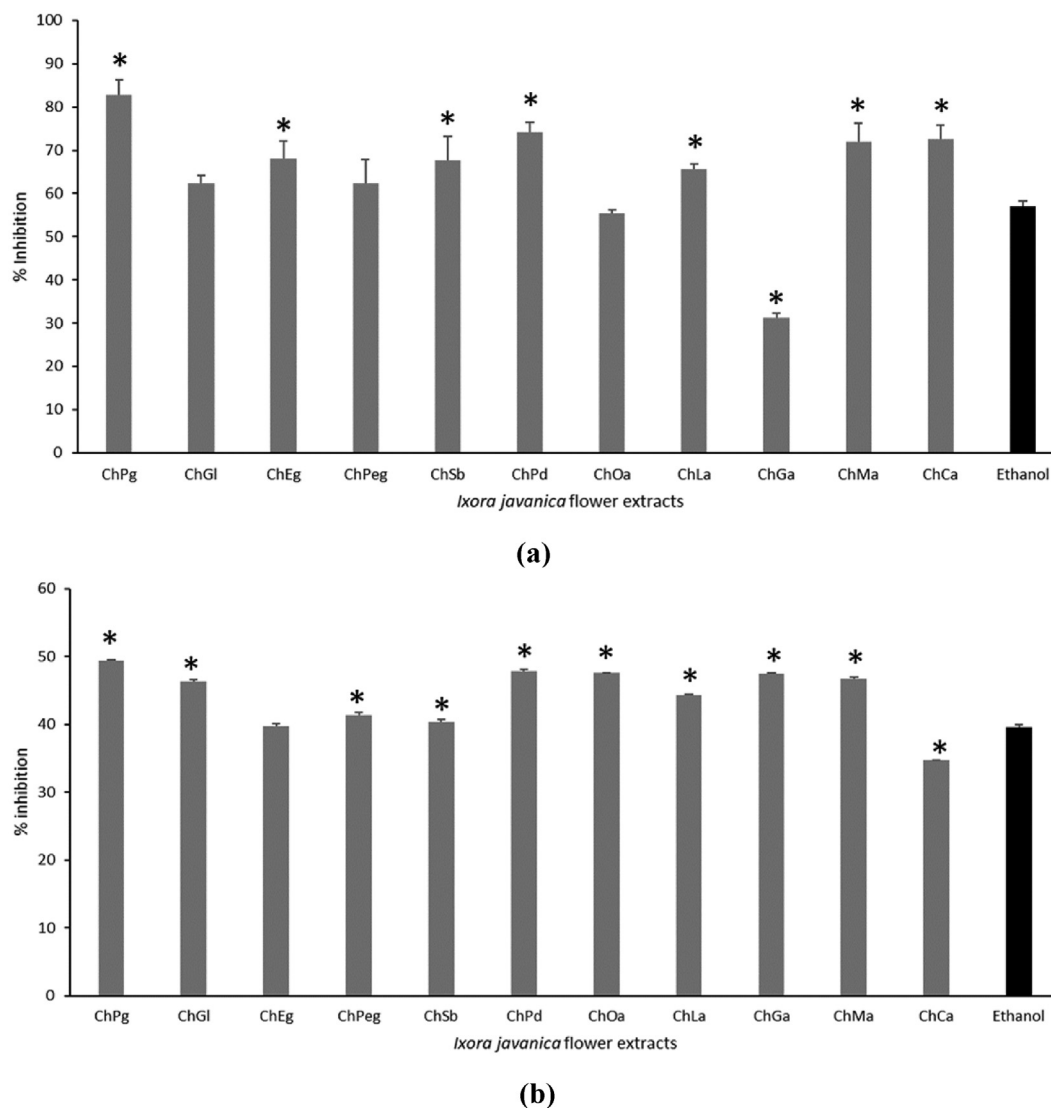


(a)



(b)

**Figure 1.** Total flavonoid (a) and anthocyanin (b) yields from *I. javanica* with different DES types. \* means  $p < 0.05$  compared with ethanol.



**Figure 2.** *In vitro* antioxidant activities (a) and tyrosinase inhibition activities (b) of *I. javanica* flower extracts obtained with various DESs. \* means  $p < 0.05$  compared with ethanol.

$$\% \text{ inhibition of tyrosinase} = \frac{(A - B) - (C - D)}{(A - B)} \times 100\% \quad (3)$$

where A is solution a absorbance; B is solution b absorbance; C is solution c absorbance; and D is solution d absorbance. Analyses were performed in triplicate.

## 2.9. Optimization of the extraction of total flavonoid using response surface methodology

Optimization of the extraction condition was assisted using response surface methodology (RSM) with three factors, where each factor consisted of three levels. RSM was done by central composite design using Minitab® Software version 16 (Minitab Pty Ltd, Sydney, Australia). Three factors were optimized to obtain a high level of total flavonoid in the flower extract. The code of each independent variable is represented in Table 3. A 20-run experiment by RSM was performed for verification and the results were compared with the predicted values.

## 2.10. Statistical analysis

Total flavonoid and TAC data are presented as the mean  $\pm$  standard deviation (SD). The results of the *in vitro* studies are expressed as the

mean of %inhibition  $\pm$ SD. All data obtained in this study were analyzed via one-way analysis of variance (ANOVA) test (significance level of  $p < 0.05$ ) using SPSS software version 16 for Windows (IBM, New York, United States).

## 3. Results and discussion

### 3.1. Flavonoid and anthocyanin extraction using different DESs

In this study, 11 DESs with different compositions and characteristics were investigated for the extraction of *I. javanica* flowers. Each DES was tested for its extraction efficiency for bioactive compounds. The results showed that different types of DESs influenced the total flavonoid and total anthocyanin yields. According to previous studies, different HBA and HBD compositions can affect the physicochemical characteristic of DESs and also their extraction efficiency (Zainal-Abidin et al., 2017). DESs containing higher polarity HBD, such as polyalcohol and acid, may be used as alternatives for flavonoid and anthocyanin extraction (Bubalo et al., 2016; Radosevic et al., 2016; Bosiljkov et al., 2017). Our results demonstrated that most of the DESs used in this study yielded better flavonoid and anthocyanin extraction than that of ethanol. Among the DESs, ChOa, ChCa, ChEg, ChPg, ChLa, ChPd, showed higher capability in flavonoid extraction compared to ethanol ( $p < 0.05$ ) (Figure 1a). While

**Table 4.** The experimental results of each variable combination.

RUN	Independent variable			Response
	X <sub>1</sub>	X <sub>2</sub>	X <sub>3</sub>	
				Total flavonoid (mg QE/g dried sample)
1	−1	1	1	33.9
2	−1	0	0	19.7
3	0	0	0	7.3
4	−1	−1	−1	17.8
5	−1	1	−1	25.9
6	1	1	−1	15.7
7	0	1	0	16.2
8	0	0	0	8.3
9	0	−1	0	8.1
10	1	−1	1	15.1
11	0	0	0	8.2
12	0	0	1	16.9
13	0	0	0	8.7
14	1	1	1	24.5
15	1	0	0	5.1
16	0	0	−1	7.6
17	0	0	0	8.5
18	0	0	0	8.7
19	1	−1	−1	4.0
20	−1	−1	1	28.4

ChEg, ChPg, ChPd demonstrated the highest anthocyanin extraction ability compared to ethanol ( $p < 0.05$ ) (Figure 1b).

Acid-based DESs provided slightly higher total levels of flavonoid than DESs with polyalcohols. Generally, acid-based DESs are more polar than sugar and polyalcohol (Craveiro et al., 2016; Radosevic et al., 2016). Based on our findings, the polarity affected the ability of the DES to extract flavonoid compounds. A similar observation that acidic and more polar DESs showed higher yields of flavonoid was reported by Duan et al. (2016). In contrast with the flavonoid extraction, polyalcohol-based DESs showed greater efficiency than acids in anthocyanin extraction. Similar results where ChPg exhibited a greater anthocyanin extraction capability than those of acid-based-DESs have been reported in previous studies (Sang et al., 2018; Meng et al., 2018).

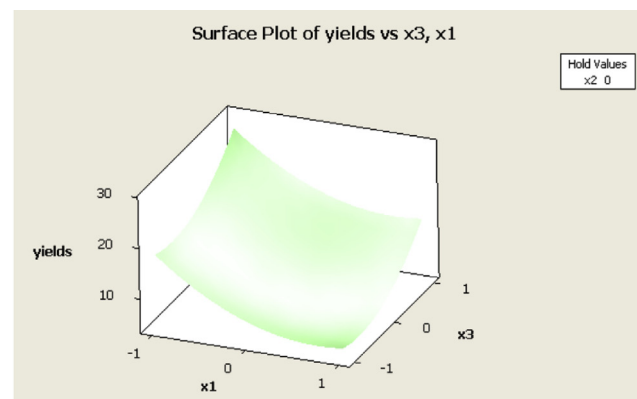
The DES's capability to extract bioactive compound is influenced by several parameters that include viscosity, polarity, solubility, and physicochemical interactions (Li et al., 2017). One possible mechanism of extraction of flavonoid compound is the formation of hydrogen bonds

**Table 5.** Analysis of variance for total flavonoid yields.

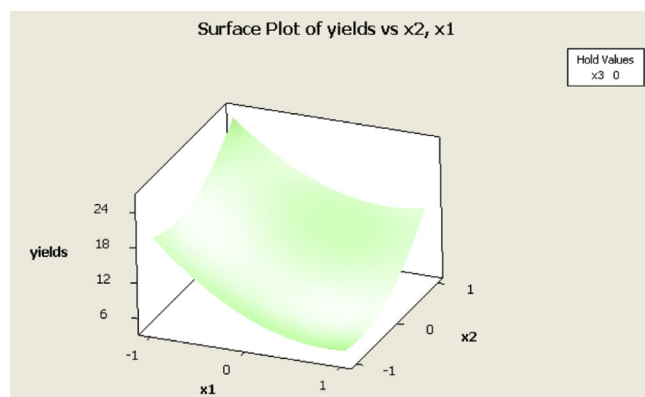
Source	Degrees of freedom	Sum of squares	Mean square	F-value	p-value
Regression	9	1363.19	151.465	299.53	0.000
Linear	3	787.44	262.479	519.07	0.000
x <sub>1</sub>	1	375.77	375.769	743.11	0.000
x <sub>2</sub>	1	183.18	183.184	362.26	0.000
x <sub>3</sub>	1	228.48	228.484	451.84	0.000
Square	3	565.50	188.502	372.78	0.000
x <sub>1</sub> *x <sub>1</sub>	1	50.20	50.205	99.28	0.000
x <sub>2</sub> *x <sub>2</sub>	1	44.50	44.501	88.00	0.000
x <sub>3</sub> *x <sub>3</sub>	1	46.74	46.741	92.43	0.000
Interaction	3	10.24	3.415	6.75	0.009
x <sub>1</sub> *x <sub>2</sub>	1	7.03	7.031	13.90	0.004
x <sub>1</sub> *x <sub>3</sub>	1	0.21	0.211	0.42	0.533
x <sub>2</sub> *x <sub>3</sub>	1	3.00	3.001	5.94	0.035
Lack-of-fit	5	3.69	0.738	2.70	0.150
Residual Error	10	5.06	0.506		
Pure Error	5	1.37	0.274		

between the DES molecules and the flavonoid (Garcia et al., 2015; Cunha and Fernandes, 2018; Liu et al., 2018). DESs with high viscosity, that can inhibit the formation of hydrogen bonds between the HBA and the HBD, had lower extraction yields in previous studies (Dai et al., 2016; Bubalo et al., 2016; Bosiljkov et al., 2017). Our results show that viscous ChSb and ChGI DESs had lower efficiency in flavonoid extraction.

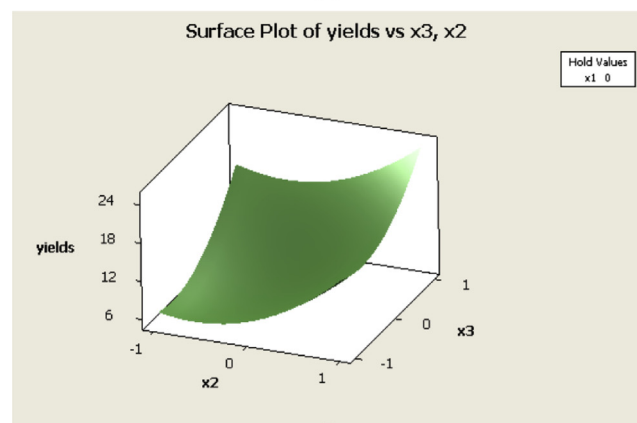
In addition, the complexity of the polyalcohol structure seems to affect the extraction efficiency of flavonoid compounds. We found that a simple structure DES component, such as ethylene glycol, resulted in greater flavonoid extraction efficiency. With more complex DES component structures, such as ChSb and ChPeg, the flavonoid yield decreased. Similar to viscosity, steric hindrance can inhibit the formation of chemical bonds, such as hydrogen, van der Waals, and hydrophobic



(a)



(b)



**Figure 3.** Interaction between extraction conditions and yield. 3D Surface graphs of (a) yield versus solid-to-liquid ratio (x3) and extraction time (x1); (b) yield versus temperature (x2) and extraction time (x1); (c) yield versus solid-to-liquid ratio (x3) and temperature (x2).



bonds, between the DES and the compound molecules (Ferrone et al., 2018). Surprisingly, this behavior did not occur with acidic DESs, possibly because the extraction mechanisms of acid-based and polyalcohol-based DESs were different.

### 3.2. Antioxidant and tyrosinase inhibition activities of the extracts

The result showed that ChPg, ChPd, ChCa, ChMa ChEg, ChSb, ChLa extracts have higher ( $p < 0.05$ ) DPPH free radical inhibition compared to ethanolic extract (Figure 2a). Statistically, ChPg extract also showed the greatest ability to neutralize DPPH free radicals among all of the extracts ( $p < 0.05$ ). Compounds with activity against free radicals and oxidative stress are a new strategy in combating aging and skin hyperpigmentation (Kanlayavattanukul et al., 2018).

Our findings show that ChPg extract was also effective as a tyrosinase inhibitor (Figure 2b). Based on statistical result, ChPg showed higher tyrosinase inhibition capability compared to all extracts ( $p < 0.05$ ). Tyrosinase is an enzyme that plays an important role in melanin production or melanogenesis. In general, three main reactions occur during the formation of melanin. First, tyrosine hydroxylation into dihydroxyphenylalanine (DOPA); second, the oxidation of DOPA to dopaquinone; and third, the oxidation of 5,6-dihydroxyindole (DHI) to indolequinone. Inhibition of tyrosinase activity will decrease melanin synthesis (Chang, 2012). Certain phenolic compounds and anthocyanin isolated from plants reportedly have tyrosinase inhibitory activity (Jhan et al., 2016). Tyrosinase inhibitors have potential not only as skin-lightening agents but also in the treatment of cancer and neurodegenerative diseases. Our results highlighted the excellent ability of ChPg as an extraction medium to produce extracts with strong antiradical effects and tyrosinase inhibition activity.

### 3.3. The optimum DES-UAE condition

The selection of the optimum DES type is the crucial point in the extraction of bioactive compounds from plants. ChPg extract showed the

highest capability as antioxidant and tyrosinase inhibitor. ChPg also significantly provided higher levels of flavonoid and anthocyanin compared to ethanol. Furthermore, ChPg was chosen as the optimum DES for *I. javanica* extraction. The extraction conditions optimized in this study included extraction time, temperature, and solid-to-liquid ratio toward total flavonoid yield as response. The responses observed in experimental results of each variable combination are presented in Table 4.

For showing the relationship between the variables and the response and also the predicted total flavonoid yields in *I. javanica* flower extract, all data was formulated in a mathematical equation model:

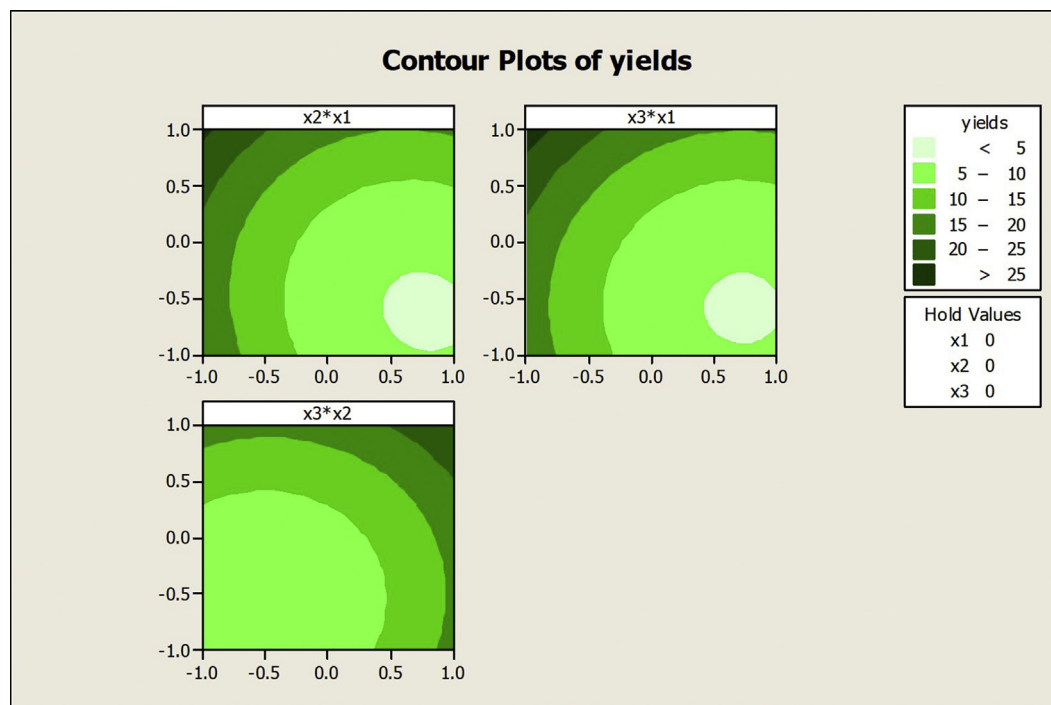
$$Y = 8.2209 - 6.1300 x_1 + 4.2800 x_2 + 4.7800 x_3 + 4.2727 x_1^2 + 4.0227 x_2^2 + 4.1227 x_3^2 + 0.9375 x_1 x_2 - 0.1625 x_1 x_3 - 0.6125 x_2 x_3$$

where  $x_1$  represents the extraction time,  $x_2$  represents the temperature, and  $x_3$  represents the solid-to-liquid ratio.

ANOVA was performed for evaluating the model quality (Table 5). The great agreement between the experimental results and the predicted yield from the model was shown by  $R^2 = 0.9779$ . This means that this model can express >97.79% of variances. The lack-of-fit showed that failure of the model in representing the data was not significant with  $p = 0.150$  ( $> 0.05$ ). The results showed that all of the variables had significant effect on the response ( $p = 0.000$ ). Interaction was seen between each of the variables ( $p < 0.05$ ) except between the extraction time and the solid-to-liquid ratio. The results are represented as contour surface and 3D surface graphs in Figure 3 and Figure 4.

The optimum point where the highest total flavonoid compound yield was obtained is shown in the dark green area in Figure 4. The extraction of the total flavonoid compound from *I. javanica* flower using ChPg reached its optimum point at an extraction time of 5 min, temperature of 57 °C and solid-to-liquid ratio of 1:50 g/mL and provided 33.9 mg QE/g dried sample. The total flavonoid yields from this study were close to the predicted value (34.1166 mg QE/g dried sample).

The extraction time is associated with the contact of the solvent with the plant material. Longer contact of the solvent with the plant material



**Figure 4.** Contour surface graphs of yields for temperature and extraction time ( $x_2 \times x_1$ ); solid-to-liquid ratio and extraction time ( $x_3 \times x_1$ ); and solid-to-liquid ratio and temperature ( $x_3 \times x_2$ ).

increased the diffusion process of the compound and thus increased the extraction efficiency. However, in UAE methods, increasing the extraction time can affect the stability of the compound. Numerous studies showed decreased extraction yields with increasing extraction time (Khezeli et al., 2016; Li et al., 2017; Syakfanaya et al., 2019).

A temperature increase in the extraction process can be an external force to increase mass transfer as well as increase solubility and diffusion and reduce viscosity. However, compound stability can be affected when high temperatures cause degradation of the compounds. Problems with high viscosity DESs in the extraction process can be solved by increasing the temperature, resulting in increased extraction efficiency (Bubalo et al., 2016; Ozturk et al., 2018; Yuniarti et al., 2019).

The solid-to-liquid ratio represents the ratio between the amount of plant material and the solvent volume in the extraction process. A lower value of the solid-to-liquid ratio means a higher volume of solvent has been added. Increasing the solvent volume can result in increasing extraction yields. However, this must be considered with the efficiency of solvents used (Ozturk et al., 2018).

#### 4. Conclusion

DESs are promising alternative green solvents that can replace the use of organic solvent for bioactive compound extraction from plants. In consideration of antioxidant activity, tyrosinase inhibitory activity, total anthocyanin and total flavonoid yield, combination of choline chloride as the HBA and propylene glycol as the HBD (molar ratio 1:1) was chosen as the solvent for extraction of *I. javanica*. Furthermore, we succeeded in optimizing the extraction process to enhance flavonoid compound in extract. The optimum extraction conditions suggested from this study were extraction time of 5 min, temperature of 57 °C, and solid-to-liquid ratio of 1:50 g/mL.

#### Declarations

##### Author contribution statement

Nina Dewi Oktaviyanti: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Kartini: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Abdul Munim: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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##### Competing interest statement

The authors declare no conflict of interest.

##### Additional information

No additional information is available for this paper.

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